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Effects of *Alangium chinense* on Motor Activity and the Concentration of Monoamines in Rats

Ming-Tsuen Hsieh*, Fu-Yu Chueh, Huei-Yann Tsai, Wen-Huang Peng and Chia-Chang Hsieh

Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, Taiwan, R. O. C.

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ABSTRACT

In this study, we used behavioral pharmacological methods to search for the sedative constitutents, except dl-anabasine, from *Alangium chinense* and to investigate their effects on motor activity and the concentration of monoamines in rat's brain. First, the fourth fraction of chloroform-A extract (CA-4 frat ion) had the most prominent sedative effect with least toxicity and did not contain the *dl*-anabasine. Second, the CA-4 fraction enhanced the hypomotility induced by α-methyl-*p*-tyrosine, reserpine and 5-hydroxytryptophan, and also reduced the hypermotility produced by levodopa plus benserazide and *p*-chlorophenylalanine. Third, the CA-4 fraction significantly decreased the concentration of norepinephrine (NE) in the cortex, dopamine (DA) and serotonin (5-HT) in the brain stem. But it increased the concentration of 5-HT in the cortex. The concentration of homovanillic acid (HVA) in the cortex and brain stem, vanillylmandelic acid (VMA) in the cortex, and 5-hydroxyindole acetic acid (5-HIAA) in the brain stem were increased by the CA-4 fraction. By contrast, the concentration of 5-HIAA in the cortex was decreased by the CA-4 fraction. These results suggest that the sedative mechanism of the CA-4 frcation might be due to the decrease in the central catecholaminergic activity and the increase in the serotonergic activity in the cortex and the decrease in the serotonergic activity in brain stem. The sedative compound of CA-4 fraction might be a triterpenoid by Lieberman büchard reaction.

Key words : *Alangium chinense*; Motor activity; Monoamines.

INTRODUCTION

Alangium chinense (Lour) Harms was described in ancient Chinese medical book for palsy, rheumatism, trauma and blood stasis[1]. In the modern pharmacological and phytochemical studies, the roots of *Alangium chinense* was found to have sedative, analgesic and muscle relax-

ant activity[2]. *DL*-Anabasine was proven to be the active constituent[2]. But it had a high toxicity (LD_{50} 10 mg/kg, p.o.)[3]. So, the purpose of the present study was intended to isolate the sedative constituents, except *dl*-anabasine, from *Alangium chinense* and tried to interpret its mechanism of sedation by behavioral pharmacological methods (e.g. motor activity and the concentration of monoamines in rats).

MATERIALS AND METHODS

Preparation of *Alangium chinese* extracts

Roots of *Alangium chinense* (L_{OUR}) H_{ARMS} (Alanginaceae) were used in our experiments. The roots were chopped, dried, extracted and partitioned as shown in Fig. 1

The chloroform-A extract was repeatedly chromatographed on column of silica gel. Elution of the column with chloroform gradually enriched with methanol provided fractions CA-1 to CA-15.

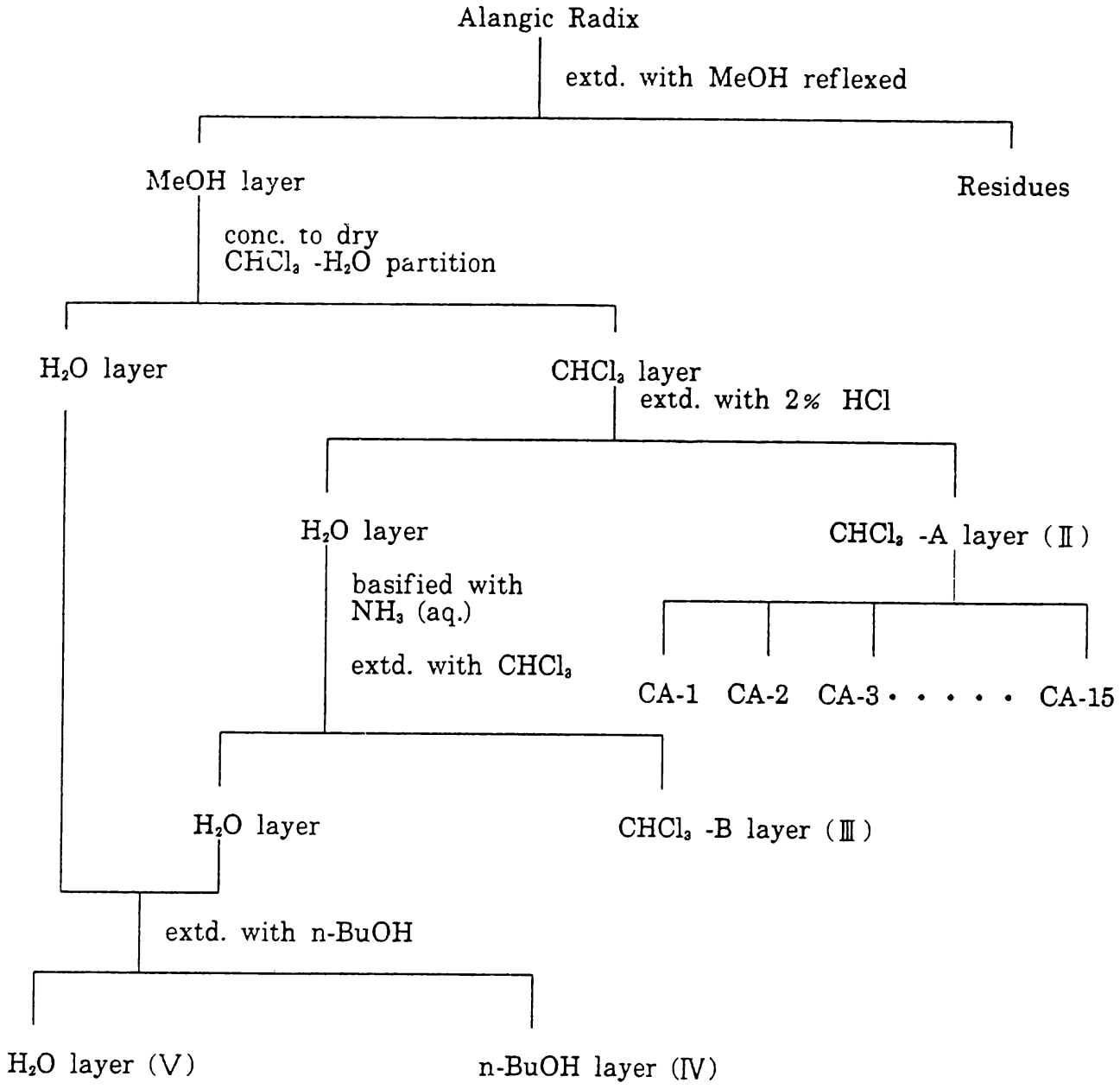

Fig. 1. Separation of *Alangium chinense* with column chromatography.

Animals

Male Sprague-Dawley rats, weighing between 200-300g, were used for the studies of motor activity and the concentration of monoamines in the brain stem and the cortex. Male ICR mice, weighing between 18-25 g, were used for the studies of hypnotic effect and muscle relaxant effect.

All animals were maintained in groups of 5 per cage at a temperaturte-control of 21-25°C and humidity of 55±5% and housed in a metal-wire mesh cage for at least 7 days before each experiment. A standard pellet diet and tap water were provided *ad libitum.*

Acute toxicity

A sufficient number of animals were used in this test. According to the method of Litchfield and Wilcoxon[4], the LD_{50} (95% confidence limits) were assessed 72 hours after oral or intraperitoneal administration of each extracts.

Hypnotic effect

Each extracts of *Alangium chinense,* except chloroform-B extract (0.1 g/kg), at a dosage of 0.5 g/kg and each fractions of chloroform-A layer at a dosage of 50 mg/kg were administrated orally to mice. Sixty minutes later, hexobarbital (100 mg/kg, i.p.) was given to induce sleep. The interval between loss and recovery of righting reflex was used as the index of the hypnotic effect[5]. Control mice received vehicle (p.o.) only.

Muscle relaxant effects

All of the *Alangium chinense* extracts, except chloroform-B extract (0.1 g/kg), at a dosage of 0.5 g/kg were administered orally to mice. The time period of hooking at the iron-wire by back-paws was used as the index of muscle relaxation and measured at a 30 mins interval of 180 mins[6]. Control mice recived the vehicle (p.o.) only.

Measurement of motor activity

The motor activity of individual rats was measured using the MK-ANIMEX activity meter (Model SE. Muromachi Kikai Co., Japan). The sensitivity and tuning of the instrument was adjusted to 35 μA to enable all kinds of motor behavior, including locomotion, rearing, grooming, sniffing and licking to be jointly recorded. Activity was recorded for 2 hours, starting 5 minutes after inserting the animal into the test cage. Each rat was used only once and totally six rats were used for each treatment[7], CA-4 fraction (50, 100 mg/kg) was administered orally. Sixty minutes later, motor activity was recorded. α-MT, 1-dopa, benserazide, reserpine, 5-HTP and PCPA were used to see their effects on the changes of motor activity produced by the CA-4 fraction. *L*-dopa, benserazide, 5-HTP and PCPA were suspended in 5% CMC. α-MT was dissolved in 0.9% saline. Reserpine was dissolved in the minimum amount of glacial acetic acid and diluted with water, the finally acetic concentration being less than 0.1%. *L*-dopa, benserazide, 5-HTP and PCPA were prepared immediately before use. The following compounds were given before administration of CA-4 fraction (50 mg/kg, p.o.) as following: α-MT (100 mg/kg, i.p.) 2 hours before; *L*-dopa (200 mg/kg, i.p.) 50 minutes before; benserazide (50 mg/kg, i.p.) 80 minutes

before; reserpine (0.5 mg/kg, i.p.) 18 hours before; 5-HTP (50 mg/kg, i.p.) 30 minutes before; PCPA (200 mg/kg, i.p.) 24 hours before. Control mice received vehicle only.

Quantification of monoamines and its metabolites by HPLC

The CA-4 fraction was given orally to rats at a dosage of 50 mg/kg. Sixty minutes later, the rats were decapitated and the whole brain was removed and separated into the cortex and brain stem. The concentration of norepinephrine, dopamine, serotonin and their metabolites (VMA, HVA, 5-HIAA) were measured by high performance liquid chromatography (HPLC) with Electrochemical Detection[8,9,10]. HPLC Model M-45 (Waters Associates), Data Model M-730 (Waters Associates) and Electrochemical Detector Model LC-4B (Bioanalytical systems) were used.

Statistics

Results were analyzed statistically using unpaired Student's *t*-test or ANOVA followed by Duncan's multiple range test to compare their differences. P value less than 0.05 was required for statistical significance.

RESULTS

Acute toxicity

Table 1 shown the LD_{50} and 95% confidence limits of various extract of *Alangium chinese*. Chloroform-A extract (>10 g/kg, p.o.) and CA-4 fraction (>10 g/kg, p.o.) had the least toxicity, but chloroform-B extract had the largest toxicity (0.64 g/kg, p.o.; 0.26 g/kg, i.p.).

Hypnotic effect

As shown in Table 2, methanol extract (6.6 ± 0.6, $p<0.05$), chloroform-A extract (5.9 ± 0.4, $p<0.05$) and water extract (5.4 ± 0.3, $p<0.01$) significantly shortened the onset and prolonged the

Table 1. LD_{50} and 95% confidence limits of *Alangium chinense* extracts in mice

Drugs	Route	LD_{50} (g/kg)	(95% confidence limits)
MeOH ext.	P.O.	4.90	(4.42-5.44 g/kg)
	I.P.	2.05	(1.76-2.38 g/kg)
$CHCl_3$-A ext.	P.O.	>10	
	I.P.	2.78	(2.61-2.96 g/kg)
$CHCl_3$-B ext.	P.O.	0 64	(0.59-0.70 g/kg)
	I.P.	0.16	(0.14-0.19 g/kg)
n-BuOH ext.	P.O.	3.50	(3.01-4.07 g/kg)
	I.P.	1.16	(1.13-1.19 g/kg)
H_2O ext.	P.O.	5.98	(5.41-6.61 g/kg)
	I.P.	3.84	(3.34-3.63 g/kg)
CA-4 fraction	P.O.	>10	
	I.P.	2.45	(1.70-3.54 g/kg)

sleeping time (56.7±4.7, 59.7±2.2 and 66.2±2.4 respectively, $p<0.01$) produced by hexobarbital. Chlorform-B extract significantly prolonged the sleeping time (48.7±3.0, $p<0.01$) only.

As shown in Table 3, CA-4 (6.50±3.8, $p<0.01$), CA-5 (43.3±5.4, $p<0.05$) and CA-15 (45.0±4.6, $p<0.05$) fractions significantly prolonged the sleeping time produced by hexobarbital. CA

Table 2. Effect of *Alangium chinense* on the hexobarbital-induced hypnosis in mice

Drugs	Dose (p.o.)	Onset (min)	Sleeping time (min)
Control	—	8.4±1.1	31.5±3.2
MeOH ext.	0.5 (g/kg)	6.6±0.6*	56.7±4.7**
$CHCl_3$-A ext.	0.5 (g/kg)	5.9±0.4*	59.7±2.2**
$CHCl_3$-B ext.	0.1 (g/kg)	9.1±0.6	48.7±3.0**
n-BuOH ext.	0.5 (g/kg)	9.4±0.5	35.3±2.2
H_2O ext.	0.5 (g/kg)	5.4±0.3**	66.2±2.4**

Mean±S.E. (n=6)
Onset: righting reflex disappear.
Sleeping time: time of onset to righting reflex recover.
*$P<0.05$, **$P<0.01$, compared with the control group (ANOVA for repeated measures followed by Duncan's multiple range test).

Table 3. Effect of CA-1 to CA-15 fractions on the hexobarbital-induced hypnosis in mice

Drugs	Dose (p.o. mg/kg)	Onset (min)	Sleeping time (min)
Control	—	8.4±0.5	31.5±2.9
CA-1	50	8.6±0.4	24.9±1.5
CA-2	50	9.0±0.6	30.6±3.2
CA-3	50	8.4±0.5	37.2±2.3
CA-4	50	8.8±0.7	65.0±3.8**
CA-5	50	8.4±0.4	43.3±5.4*
CA-6	50	9.4±0.9	41.4±3.2
CA-7	50	9.1±0.8	41.6±1.8
CA-8	50	9.9±0.4	33.3±2.3
CA-9	50	7.4±0.2	36.1±4.0
CA-10	50	6.6±0.3*	32.7±3.3
CA-11	50	8.1±0.6	28.6±5.0
CA-12	50	7.2±1.0	31.9±2.3
CA-13	50	8.1±0.7	28.8±2.9
CA-14	50	8.2±0.8	41.5±4.7
CA-15	50	8.4±1.0	45.0±4.6*

Mean±S.E. (n=6)
Onset: righting reflex disappear.
Sleeping time: time of onset to righting reflex recover.
*$P<0.05$, **$P<0.01$, compared with the control group (ANOVA for repeated measures followed by Duncan's multiple range test).

-10 fraction (6.6±0.3, $p<0.05$) shorten the onset produced by hexobarbital.

Muscle relaxant effects

As shown in Table 4, chloroform-B extract and *n*-butanol extract had the strongest muscle relaxant effect. Methanol extract only had a weak muscle relaxant effect.

Table 4. Effect of *Alangium chinense* extracts on traction performance in mice

Drugs	Dose (g/kg) p.o.	No. of mice treated	No. of mice which fell within 5 seconds after drug administration			
			30	60	90	120 (min)
Control	—	6	0	0	0	0
MeOH ext.	0.5	6	2	3	2	1
$CHCl_3$-A ext.	0.5	6	1	1	0	0
$CHCl_3$-B ext.	0.1	6	2	2	1	2
n-BuOH ext.	0.5	6	2	4	3	3
H_2O ext.	0.5	6	0	1	2	2
CA-4 fraction	0.05	6	0	0	0	0

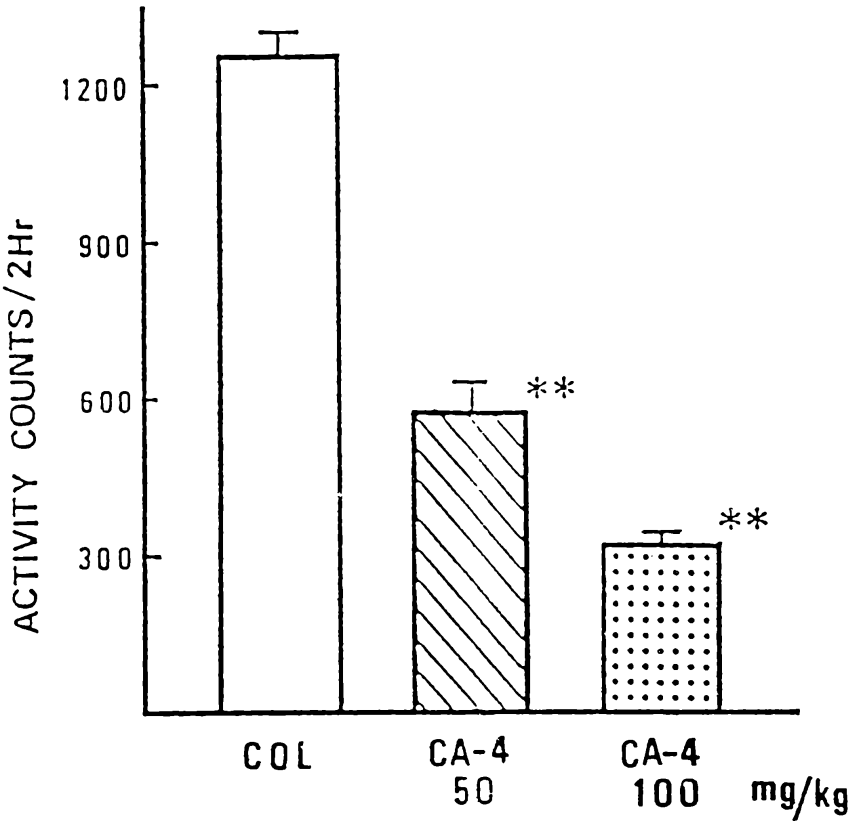

Fig. 2. Effects of the CA-4 fraction (50 mg/kg, 100 mg/kg) on the locomotr activity in rats.
COL=control.
CA-4 =CA-4 fraction 1 hr prior to testing.
Values are means±S.E. n= 6.
**represents statistically significant as compared to control ($P<0.01$)

Effect on motor activity

As shown in Fig. 2, the CA-4 fraction produced a sinificant decrease in motor activity at 50 mg/kg (598±54, $p<0.01$) and 100 mg/kg (302±20, $p<0.01$) and shown dose-dependent response.

L-dopa plus benserazide produced a significant increase in motor acitivity (4000±150). The hypermotility produced by *l*-dopa plus benserazide was significantly reduced by the CA-4 fraction (2000±148, $p<0.01$) (Fig. 3). Rats treated with α-MT (1001±150, $p<0.01$) or reserpine

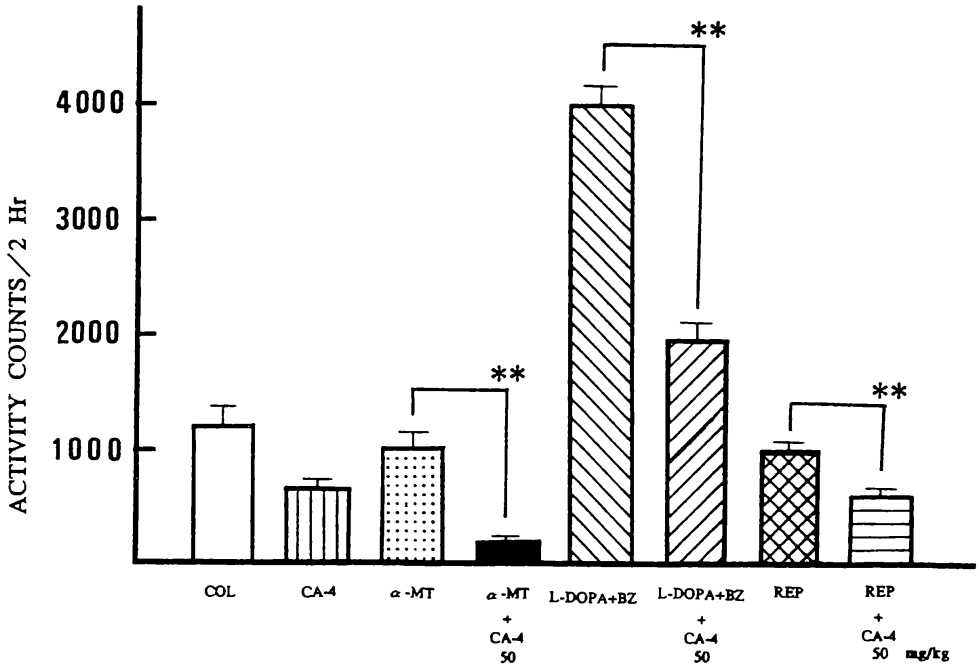

Fig. 3. Effects of the CA-4 fraction (50 mg/kg) on the locomotor activity produced by α-methyl-*p*-tyrosine (α-MT; 50 mg/kg), *l*-dopa (200 mg/kg) + benserazide (50 mg/kg) and reserpine (0.5 mg/kg) in rats.
COL=control.
Values are means±S.E., ** $P<0.01$.

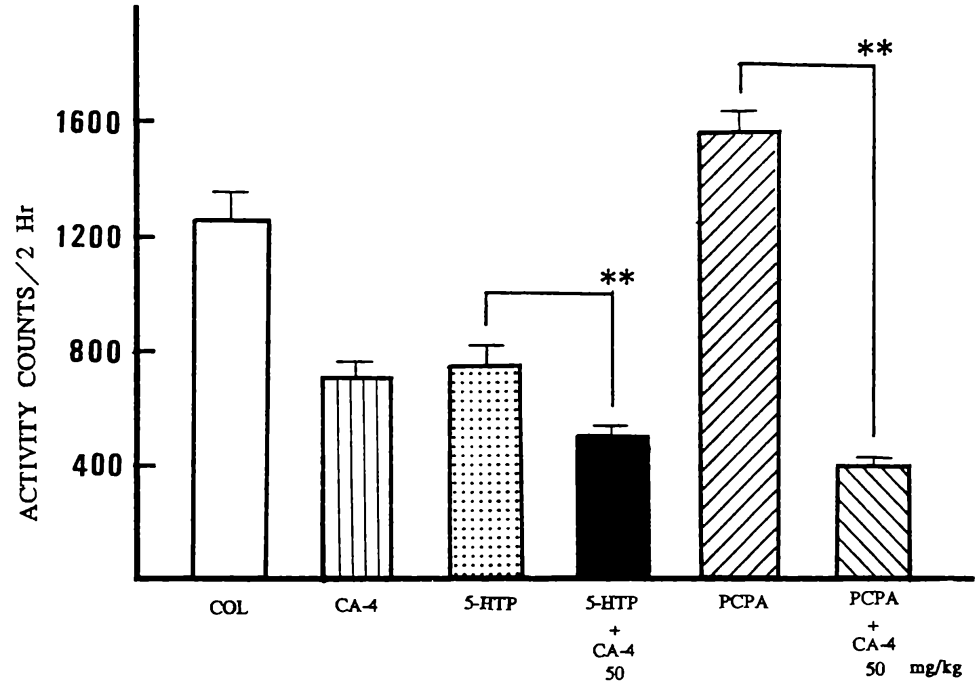

Fig. 4. Effects of the CA-4 fraction (50 mg/kg) on the locomotor activity produced by 5-hydroxytryptophan (5-HTP; 50 mg/kg) and *p*-chlorophenylanine (PCPA; 200 mg/kg) in rats.
COL=control.
Values are means±S.E., ** $P<0.01$.

Table 5. Effects of CA-4 fraction on monoamines and metabolites in cortex and brain stem in rat brain

	cortex (ng/g)		brain stem (ng/g)	
	control	CA-4	control	CA-4
NE	3775.1±122.3	2498.5±155.8***	4419.2±682.5	3194.5±171.9
DA	2546.8±176.9	2294.1±203.7	3462.1±672.5	1849.1±161.9*
5-HT	865.0±83.2	2004.2±196.4***	1564.4±176.7	674.2±109.8***
VMA	216.6±18.6	448.1±51.2**	392.2±48.0	428.7±30.9
HVA	124.6±14.2	228.4±45.1*	88.1±26.1	266.7±35.0**
5-HIAA	237.2±18.9	113.5±10.8***	196.0±11.1	408.3±42.3**

Mean±S.E. (n=5)
*P<0.05, **P<0.01, ***P<0.001, compared with control group by Student's *t*-test.

(1000±51, $p<0.01$) appeared to be behavioral depressed. The hypomotilities produced by α-MT or reserpine were significantly augmented by CA-4 fraction (160±140 and 575±55 respectively, $p<0.01$) (Fig. 3).

Rats treated with 5-HTP also appeared to be behaviorally depressed (720±64). The hypomotility produced by 5-HTP was significantly augmented by the CA-4 fraction (480±24, $p<0.01$) (Fig. 4). PCPA produced a significant increase in motor activity (1520±62). The hypermotility procduced by PCPA was significantly reduced by CA-4 fraction (380±20, $p<0.01$) (Fig. 4).

Effects on the concentration of monoamines and its metabolites in the cortex and the brain stem

As shown in Table 5, the CA-4 fraction significantly decreased the level of NE (2498.5±155.8, $p<0.001$) and 5-HIAA (113.5±10.8, $p<0.001$) in the cortex, DA (1849.1±161.9, $p<0.05$) and 5-HT (674.2±109.8, $p<0.01$) in the brain stem. It significantly increased the level of 5-HT (2004.2±196.4, $p<0.001$), VMA (448.1±51.2, $p<0.01$) and HVA (228.4±45.1, $p<0.05$) in the cortex, HVA (266.7±35.0, $p<0.01$) and 5-HIAA (408.3±42.3, $p<0.01$) in the brain stem.

DISCUSSION

In the present study, the total methanol extract of the roots of *Alangium chinense* was partitioned into chloroform-A, chlororfrorm-B, *n*-butanol and water extracts. Chloroform-A extract significantly prolonged the hexobarbital-induced sleeping time. It did not contain *dl*-anabasine and have low toxicity. Therefore, the chloroform-A extract was separated into 15 fractions (CA-1~CA-15) by column chromatography and evaluated their sedative effect by hexobarbital-induced sleeping time. The CA-4 fraction had the strongest sedative effect and did not contain *dl*-anabasine.

First, the CA-4 fraction significantly decreased motor activity, but it had no muscle relaxation. These facts implied that the sedative effect of CA-4 fraction may be related to the inhibition of the CNS.

α-MT (an inhibitor of tyrosine hydroxylase) pretreatment depletes the newly-synthesized pool of catecholamines[11], whereas reserpine depletes the stored-vesicular pool of CNS animes (e.g. catecholamine, serotonin)[12,13]. The two compounds can reduce motor activity[14,15,16]. Levodopa (a precursor of dopamine) plus benserazide (a dopa decarboxylase inhibitor)[17] can produced large increases in motor activity. The CA-4 fractoin significantly augmented the hypomotilities produced by α-MT and reserpine, and reduce the hypermotilities produced by *l*-dopa plus benserazide. These facts implied that CA-4 fraction decreased the catecholaminergic activity. From the studies on the concentration of monoamines in rat's brain, we further found that CA-4 fraction significantly decreased the concentration of NE and increased the concentration of VMA, HVA in the cortex. It also significantly decreased the concentration of DA and increased the concentration of HVA in the brain stem. Therefore, the sedative effect of the CA-4 fraction may be related to the decreases in the concentration of NE and DA, and the increase in the turnover rate of NE and DA in the brain stem and the cortex.

5-HTP (a precursor of serotonin) can increase the central 5-HT level with a subsequent decrease in motor activity[18]. The CA-4 fractin augmented the hypomotility induced by 5-HTP. PCPA (a serotonin synthesis inhibitor) can cause catecholamine arousal with a subsequent increase in motor activity[19]. The CA-4 fraction could depress the hypermotility induced by PCPA. This fact implied that CA-4 fraction decreased the catecholaminergic activity more than serotonergic activity. Both of them were no statistical significance. From the studies on the concentration of monoamines in rats, we found that CA-4 fraction significantly increased the concentration of 5-HT and decreased the concentration of 5-HIAA in the cortex, but it decreased the concentration of 5-HT and increased the concentration of 5-HIAA in the brain stem. Generally, serotonin has an inhibitory action on CNS neurons, while facilitating the excitation of motor neuron in the brain stem and spinal cord[20]. So, the effect of the CA-4 fraction on serotonergic activity may be related to the increase in the concentration of 5-HT and the decreae in the turnover rate of 5-HT in the cortex, and the decrease in the concentration of 5-HT and the increase in the turnover rate of 5-HT in the brain stem.

In conclusion, the sedative mechanism of CA-4 fraction may be related to the decrease in the central catecholaminergic activity and increase in the serotonergic activity in the cortex and decrease in the serotonergic activity in the brain stem.

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中 文 摘 要

八角楓分離物對大鼠自發運動量及腦內單胺濃度之影響

謝明村　闕甫仔　蔡輝彥　彭文煌　謝佳璋

臺中市　中國醫藥學院　中國藥學研究所

八角楓〔*Alangium chinense* ($L_{OUR.}$)H_{ARMS}〕爲民間廣泛用於治療風濕痛、勞傷腰痛、筋骨疼痛及跌打損傷等鎮痛之要藥。本研究以行爲藥理學的方法，尋找八角楓根部非八角楓鹼之鎮靜有效成分，探討其對於中樞神經系統之作用與腦內單胺濃度之影響。研究結果顯示：八角楓氯仿-A 層之 CA－4 分離物鎮靜效果最強、毒性最小且不含八角楓已知成分八角楓鹼(*dl*-anabasine)。CA-4 分離物能增強由 α-甲基對酪胺酸、蛇根鹼及 5-羥基色胺酸誘發之運動量抑制作用；CA-4 分離物亦能抑制由 *l*-多巴加上苯絲胺醯肼及對氯代苯丙胺酸所誘發之運動興奮作用。而其鎮靜作用機轉可能與抑制中樞兒茶胺性系統及腦幹中血清緊素性系統活性之作用有關，且與增強大腦皮質之血清緊素性系統活性之作用有關。